DESCRIPTION

PROCESS FOR ALTERING THE HOST RANGE OF BACILLUS THURINGIENSIS TOXINS, AND NOVEL TOXINS PRODUCED THEREBY

CROSS REFERENCE TO A RELATED APPLICATION

This is a continuation-in-part of our copending application Serial No. 808,129, filed on December 12, 1985.

Background of the Invention

The most widely used microbial pesticides are derived from the bacterium Bacillus thuringiensis. This bacterial agent is used to control a wide range of leaf-eating caterpillars, Japanese beetles and mosquitos. Bacillus thuringiensis produces a proteinaceous paraspore or crystal which is toxic upon ingestion by a susceptible insect host. For example, B. thuringiensis var. kurstaki HD-1 produces a crystal called a delta toxin which is toxic to the larvae of a number of lepidopteran insects. The cloning and expression of this B.t. crystal protein gene in Escherichia coli has been described in the published literature (Schnepf, H.E. and Whiteley, H.R. [1981] Proc. Natl. Acad. Sci. USA 78:2893-2897). U.S. Patent 4,448,885 and U.S. Patent 4,467,036 both disclose the expression of B.t. crystal protein in E. coli. In U.S. 4,467,036 B. thuringiensis var. kurstaki HD-1 is disclosed as being available from the well-known NRRL culture repository at Peoria, Illinois. Its accession number there is NRRL B-3792. B. thuringiensis var. kurstaki HD-73 is also available from NRRL. Its accession number is NRRL B-4488.

Brief Summary of the Invention

The subject invention concerns a novel process for altering the insect host range of Bacillus thuringiensis toxins, and novel toxins produced as exemplification of this useful process. This alteration can result in expansion of the insect host range of the toxin, and/or, amplification of host toxicity. The process comprises recombining in vitro the variable region(s) of two or more δ-endotoxin genes. Specifically exemplified is the recombining of portions of two Bacillus thuringiensis var. kurstaki DNA sequences, i.e., referred to herein as k-1 and k-73, to produce chimeric B.t. toxins with expanded host ranges as compared to the toxins produced by the parent DNA's.

"Variable regions," as used herein, refers to the non-homologous regions of two or more DNA sequences. As shown by the examples presented herein, the recombining of such variable regions from two different B.t. DNA sequences yields, unexpectedly, a DNA sequence encoding a δ-endotoxin with an expanded insect host range. In a related example, the recombining of two variable regions of two different B.t. toxin genes results in the creation of a chimeric toxin molecule with increased toxicity toward the target insect. The utility of this discovery by the inventors is clearly broader than the examples disclosed herein. From this discovery, it can be expected that a large number of new and useful toxins will be produced. Thus, though the subject process is exemplified by construction of chimeric toxin-producing DNA sequences from two well-known B.t. kurstaki DNA sequences, it should be understood that the process

is not limited to these starting DNA sequences. The invention process also can be used to construct chimeric toxins from any B. thuringiensis toxin-producing DNA sequence.

Description of the Drawings

FIGURE 1: A schematic diagram of plasmid pEW1 which contains the DNA sequence encoding Bacillus thuringiensis toxin k-1.

FIGURE 2: A schematic diagram of plasmid pEW2 which contains the DNA sequence encoding Bacillus thuringiensis toxin k-73.

FIGURE 3: A schematic diagram of plasmid pEW3 which contains the DNA sequence encoding Bacillus thuringiensis chimeric toxin k-73/k-1 (pHY).

FIGURE 4: A schematic diagram of plasmid pEW4 which contains the DNA sequence encoding Bacillus thuringiensis chimeric toxin k-1/k-73 (pYH).

Detailed Disclosure of the Invention

Upon recombining in vitro the variable region(s) of two or more δ-endotoxin genes, there is obtained a gene(s) encoding a chimeric toxin(s) which has an expanded and/or amplified host toxicity as compared to the toxin produced by the starting genes. This recombination is done using standard well-known genetic engineering techniques.

The restriction enzymes disclosed herein can be purchased from Bethesda Research Laboratories, Gaithersburg, MD, or New England Biolabs, Beverly, MA. The enzymes are used according to the instructions provided by the supplier.

The various methods employed in the preparation of the plasmids and transformation of host organisms are well known in the art. These procedures are all described in Maniatis, T., Fritsch, E.F., and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York. Thus, it is within the skill of those in the genetic engineering art to extract DNA from microbial cells, perform restriction enzyme digestions, electrophorese DNA fragments, tail and anneal plasmid and insert DNA, ligate DNA, transform cells, prepare plasmid DNA, electrophorese proteins, and sequence DNA.

Plasmids pEW1, pEW2, pEW3, and pEW4, constructed as described infra, have been deposited in E. coli hosts in the permanent collection (to be maintained for at least 30 years) of the Northern Regional Research Laboratory (NRRL), U.S. Department of Agriculture, Peoria, Illinois, USA. Their accession numbers and dates of deposit are as follows:

pEW1--NRRL B-18032; deposited on Nov. 29, 1985
pEW2--NRRL B-18033; deposited on Nov. 29, 1985
pEW3--NRRL B-18034; deposited on Nov. 29, 1985
pEW4--NRRL B-18035; deposited on Nov. 29, 1985

B. thuringiensis strain MTX-36, NRRL B-18101 was deposited on August 25, 1986.

Plasmid pBR322 is a well-known and available plasmid. It is maintained in the E. coli host ATCC 37017. Purified pBR322 DNA can be obtained as described in Bolivar, F., Rodriguez, R.L., Greene, P.J., Betlach, M.C., Heynecker, H.L., Boyer, H.W., Crosa, J.H. and Falkow, S. (1977) Gene 2:95-113; and Sutcliffe, J.G. (1978) Nucleic Acids Res. 5:2721-2728.

NRRL B-18032, NRRL B-18033, NRRL B-18034, NRRL B-18035, and NRRL B-18101 are available to the public upon the grant of a patent which discloses these accession

numbers in conjunction with the invention described herein. It should be understood that the availability of these deposits does not constitute a license to practice the subject invention in derogation of patent rights granted for the subject invention by governmental action.

As disclosed above, any B. thuringiensis toxin-producing DNA sequence can be used as starting material for the subject invention. Examples of B. thuringiensis organisms, other than those previously given, are as follows:

Bacillus thuringiensis var. israelensis--ATCC 35646
Bacillus thuringiensis M-7--NRRL B-15939
Bacillus thuringiensis var. tenebrionis--DSM 2803

The following B. thuringiensis cultures are available from the United States Department of Agriculture (USDA) at Brownsville, Texas. Requests should be made to Joe Garcia, USDA, ARS, Cotton Insects Research Unit, P.O. Box 1033, Brownsville, Texas 78520 USA.

B. thuringiensis HD2
B. thuringiensis var. finitimus HD3
B. thuringiensis var. alesti HD4
B. thuringiensis var. kurstaki HD73
B. thuringiensis var. sotto HD770
B. thuringiensis var. dendrolimus HD7
B. thuringiensis var. kenyae HD5
B. thuringiensis var. galleriae HD29
B. thuringiensis var. canadensis HD224
B. thuringiensis var. entomocidus HD9
B. thuringiensis var. subtoxicus HD109
B. thuringiensis var. aizawai HD11
B. thuringiensis var. morrisoni HD12
B. thuringiensis var. ostriniae HD501
B. thuringiensis var. tolworthi HD537

B. thuringiensis var. darmstadiensis HD146
B. thuringiensis var. toumanoffi HD201
B. thuringiensis var. kyushuensis HD541
B. thuringiensis var. thompsoni HD542
B. thuringiensis var. pakistani HD395
B. thuringiensis var. israelensis HD567
B. thuringiensis var. indiana HD521
B. thuringiensis var. dakota
B. thuringiensis var. tohokuensis HD866
B. thuringiensis var. kumanotoensis HD867
B. thuringiensis var. tochigiensis HD868
B. thuringiensis var. colmeri HD847
B. thuringiensis var. wuhanensis HD525

Though the main thrust of the subject invention is directed toward a process for altering the host range of B. thuringiensis toxins, the process is also applicable in the same sense to other Bacillus toxin-producing microbes. Examples of such Bacillus organisms which can be used as starting material are as follows:

Bacillus cereus--ATCC 21281
Bacillus moritai--ATCC 21282
Bacillus popilliae--ATCC 14706
Bacillus lentimorbus--ATCC 14707
Bacillus sphaericus--ATCC 33203

Bacillus thuringiensis M-7, exemplified herein, is a Bacillus thuringiensis isolate which, surprisingly, has activity against beetles of the order Coleoptera but not against Trichoplusia ni, Spodoptera exigua or Aedes aegypti. Included in the Coleoptera are

various Diabrotica species (family Chrysomelidae) that are responsible for large agricultural losses, for example, D. undecimpunctata (western spotted cucumber beetle), D. longicornis (northern corn rootworm), D. virgitera (western corn rootworm), and D. undecimpunctata howardi (southern corn rootworm).

B. thuringiensis M-7 is unusual in having a unique parasporal body (crystal) which under phase contrast microscopy is dark in appearance with a flat, square configuration.

The pesticide encoded by the DNA sequence used as starting material for the invention process can be any toxin produced by a microbe. For example, it can be a polypeptide which has toxic activity toward a eukaryotic multicellular pest, such as insects, e.g., coleoptera, lepidoptera, diptera, hemiptera, dermaptera, and orthoptera; or arachnids; gastropods; or worms, such as nematodes and platyhelminths. Various susceptible insects include beetles, moths, flies, grasshoppers, lice, and earwigs.

Further, it can be a polypeptide produced in active form or a precursor or proform requiring further processing for toxin activity, e.g., the novel crystal toxin of B. thuringiensis var. kurstaki, which requires processing by the pest.

The constructs produced by the process of the invention, containing chimeric toxin-producing DNA sequences, can be transformed into suitable hosts by using standard procedures. Illustrative host cells may include either prokaryotes or eukaryotes,

normally being limited to those cells which do not produce substances toxic to higher organisms, such as mammals. However, organisms which produce substances toxic to higher organisms could be used, where the toxin is unstable or the level of application sufficiently low as to avoid any possibility of toxicity to a mammalian host. As hosts, of particular interest will be the prokaryotes and lower eukaryotes, such as fungi. Illustrative prokaryotes, both Gram-negative and -positive, include Enterobacteriaceae, such as Escherichia, Erwinia, Shigella, Salmonella, and Proteus; Bacillaceae; Rhizobiaceae, such as Rhizobium; Spirillaceae, such as photobacterium, Zymomonas, Serratia, Aeromonas, Vibrio, Desulfovibrio, Spirillum; Lactobacillaceae; Pseudomonadaceae, such as Pseudomonas and Acetobacter; Azotobacteraceae and Nitrobacteraceae. Among eukaryotes are fungi, such as Phycomycetes and Ascomycetes, which includes yeast, such as Saccharomyces and Schizosaccharomyces; and Basidiomycetes yeast, such as Rhodotorula, Aureobasidium, Sporobolomyces, and the like.

Characteristics of particular interest in selecting a host cell for purposes of production include ease of introducing the chimeric toxin-producing gene into the host, availability of expression systems, efficiency of expression, stability of the pesticide in the host, and the presence of auxiliary genetic capabilities. Characteristics of interest for use as a pesticide microcapsule include protective qualities for the pesticide,

such as thick cell walls, pigmentation, and intracellular packaging or formation of inclusion bodies; leaf affinity; lack of mammalian toxicity; attractiveness to pests for ingestion; ease of killing and fixing without damage to the toxin; and the like. Other considerations include ease of formulation and handling, economics, storage stability, and the like.

Host organisms of particular interest include yeast, such as Rhodotorula sp., Aureobasidium sp., Saccharomyces sp., and Sporobolomyces sp.; phylloplane organisms such Pseudomonas sp., Erwinia sp. and Flavobacterium sp.; or such other organisms as Escherichia, Lactobacillus sp., Bacillus sp., and the like. Specific organisms include Pseudomonas aeruginosa, Pseudomonas fluorescens, Saccharomyces cerevisiae, Bacillus thuringiensis, Escherichia coli, Bacillus subtilis, and the like.

The chimeric toxin-producing gene(s) can be introduced into the host in any convenient manner, either providing for extrachromosomal maintenance or integration into the host genome.

Various constructs may be used, which include replication systems from plasmids, viruses, or centromeres in combination with an autonomous replicating segment (ars) for stable maintenance. Where only integration is desired, constructs can be used which may provide for replication, and are either transposons or have transposon-like insertion activity or provide for homology with the genome of the host. DNA sequences can be employed having the chimeric toxin-producing gene between sequences which are homologous with sequences in the genome of the host, either chromosomal or plasmid. Desirably, the chimeric toxin-producing gene(s) will be present in multiple copies.

See for example, U.S. Patent No. 4,399,216. Thus, conjugation, transduction, transfection and transformation may be employed for introduction of the gene.

A large number of vectors are presently available which depend upon eukaryotic and prokaryotic replication systems, such as ColE1, P-1 incompatibility plasmids, e.g., pRK290, yeast 2m μ plasmid, lambda, and the like.

Where an extrachromosomal element is employed, the DNA construct will desirably include a marker which allows for a selection of those host cells containing the construct. The marker is commonly one which provides for biocide resistance, e.g., antibiotic resistance or heavy metal resistance, complementation providing prototrophy to an auxotrophic host, or the like. The replication systems can provide special properties, such as runaway replication, can involve cos cells, or other special feature.

Where the chimeric toxin-producing gene(s) has transcriptional and translational initiation and termination regulatory signals recognized by the host cell, it will frequently be satisfactory to employ those regulatory features in conjunction with the gene. However, in those situations where the chimeric toxin-producing gene is modified, as for example, removing a leader sequence or providing a sequence which codes for the mature form of the pesticide, where the entire gene encodes for a precursor, it will frequently be necessary to manipulate the DNA sequence, so that a transcriptional initiation regulatory sequence may be provided which is different from the natural one.

A wide variety of transcriptional initiation sequences exist for a wide variety of hosts. The sequence can provide for constitutive expression of the

pesticide or regulated expression, where the regulation may be inducible by a chemical, e.g., a metabolite, by temperature, or by a regulatable repressor. See for example, U.S. Patent No. 4,374,927. The particular choice of the promoter will depend on a number of factors, the strength of the promoter, the interference of the promoter with the viability of the cells, the effect of regulatory mechanisms endogenous to the cell on the promoter, and the like. A large number of promoters are available from a variety of sources, including commercial sources.

The cellular host containing the chimeric toxin-producing pesticidal gene may be grown in any convenient nutrient medium, where the DNA construct provides a selective advantage, providing for a selective medium so that substantially all or all of the cells retain the chimeric toxin-producing gene. These cells may then be harvested in accordance with conventional ways and modified in the various manners described above. Alternatively, the cells can be fixed prior to harvesting.

Host cells transformed to contain chimeric toxin-producing DNA sequences can be treated to prolong pesticidal activity when the cells are applied to the environment of a target pest. This treatment can involve the killing of the host cells under protease deactivating or cell wall strengthening conditions, while retaining pesticidal activity.

The cells may be inhibited from proliferation in a variety of ways, so long as the technique does not deleteriously affect the properties of the pesticide, nor diminish the cellular capability in protecting

the pesticide. The techniques may involve physical treatment, chemical treatment, changing the physical character of the cell or leaving the physical character of the cell substantially intact, or the like.

Various techniques for inactivating the host cells include heat, usually 50°C to 70°C; freezing; UV irradiation; lyophilization; toxins, e.g., antibiotics; phenols; anilides, e.g., carbanilide and salicylanilide; hydroxyurea; quaternaries; alcohols; antibacterial dyes; EDTA and amidines; non-specific organic and inorganic chemicals, such as halogenating agents, e.g., chlorinating, brominating or iodinating agents; aldehydes, e.g., glutaraldehyde or formaldehyde; toxic gases, such as ozone and ethylene oxide; peroxide; psoralens; desiccating agents; or the like,which may be used individually or in combination. The choice of agent will depend upon the particular pesticide, the nature of the host cell, the nature of the modification of the cellular structure, such as fixing and preserving the cell wall with cross-linking agents, or the like.

The cells generally will have enhanced structural stability which will enhance resistance to environmental degradation in the field. Where the pesticide is in a proform, the method of inactivation should be selected so as not to inhibit processing of the proform to the mature form of the pesticide by the target pest pathogen. For example, formaldehyde will crosslink proteins and could inhibit processing of the proform of a polypeptide pesticide. The method of inactivation or killing retains at least a substantial portion of the bioavailability or bioactivity of the toxin.

The method of treating the organism can fulfill a number of functions. First, it may enhance structural integrity. Second, it may provide for enhanced proteolytic stability of the toxin, by modifying the toxin so as to reduce its susceptibility to proteolytic degradation and/or by reducing the proteolytic activity of proteases naturally present in the cell. The cells are preferably modified at an intact stage and when there has been a substantial build-up of the toxin protein. These modifications can be achieved in a variety of ways, such as by using chemical reagents having a broad spectrum of chemical reactivity. The intact cells can be combined with a liquid reagent medium containing the chemical reagents, with or without agitation at temperatures in the range of about -10 to 60°C. The reaction time may be determined empirically and will vary widely with the reagents and reaction conditions. Cell concentrations will vary from about 10E2 to 10E10 per ml.

Of particular interest as chemical reagents are halogenating agents, particularly halogens of atomic no. 17-80. More particularly, iodine can be used under mild conditions and for sufficient time to achieve the desired results. Other suitable techniques include treatment with aldehydes, such as formaldehyde and glutaraldehyde; anti-infectives, such as zephiran chloride and cetylpyridinium chloride; alcohols, such as isopropyl and ethanol; various histologic fixatives, such as Bouin's fixative and Helly's fixative (See: Humason, Gretchen L., Animal Tissue Techniques, W.H. Freeman and Company, 1967); or a combination of physical (heat) and chemical agents that prolong the activity of the toxin produced in the cell when the cell is applied to the environment of the target pest(s).

For halogenation with iodine, temperatures will generally range from about 0 to 50°C, but the reaction can be conveniently carried out at room temperature.

Conveniently, the iodination may be performed using triiodide or iodine at 0.5 to 5% in an acidic aqueous medium, particularly an aqueous carboxylic acid solution that may vary from about 0.5-5M. Conveniently, acetic acid may be used, although other carboxylic acids, generally of from about 1 to 4 carbon atoms, may also be employed. The time for the reaction will generally range from less than a minute to about 24 hrs, usually from about 1 to 6 hrs. Any residual iodine may be removed by reaction with a reducing agent, such as dithionite, sodium thiosulfate, or other reducing agent compatible with ultimate usage in the field. In addition, the modified cells may be subjected to further treatment, such as washing to remove all of the reaction medium, isolation in dry form, and formulation with typical stickers, spreaders, and adjuvants generally utilized in agricultural applications, as is well known to those skilled in the art.

Of particular interest are reagents capable of crosslinking the cell wall. A number of reagents are known in the art for this purpose. The treatment should result in enhanced stability of the pesticide. That is, there should be enhanced persistence or residual activity of the pesticide under field conditions. Thus, under conditions where the pesticidal activity of untreated cells diminishes, the activity of treated cells remains for periods of from 1 to 3 times longer.

The cells can be formulated for use in the environment in a variety of ways. They can be employed as wettable powders, granules, or dusts, by mixing with various inert materials, such as inorganic minerals (phyllosilicates, carbonates, sulfates, or phosphates)

or botanical materials (powdered corncobs, rice hulls, or walnut shells). The formulations can include spreader/sticker adjuvants, stabilizing agents, other pesticidal additives, or surfactants. Liquid formulations can be aqueous-based or non-aqueous and employed as foams, gels, suspensions, emulsifiable concentrates, and the like. The ingredients can include rheological agents, surfactants, emulsifiers, dispersants, polymers, and the like.

The pesticidal concentration will vary depending upon the nature of the particular formulation, e.g., whether it is a concentrate or to be used undiluted. The pesticide will generally be present at a concentration of at least about 1% by weight, but can be up to 100% by weight. The dry formulations will have from about 1 to 95% by weight of the pesticide, while the liquid formulations will generally be from about 1 to 60% by weight of the solids in the liquid phase. The formulations will generally have from about 1E2 to 1E8 cells/mg.

The formulations can be applied to the environment of the pest(s), e.g., plants, soil or water, by spraying, dusting, sprinkling, or the like. These formulations can be administered at about 2 oz (liquid or dry) to 2 or more pounds per hectare, as required.

Following are examples which illustrate procedures, including the best mode, for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

Example 1--Construction of plasmid pEW1

The k-1 gene is the hd-1 gene described by Schnepf et al. (J. Biol. Chem. 260:6264-6272 1985).

The k-1 gene was resected from the 5' end with Bal31 up to position 504. To this position was added a SalI linker (5'GTCGACC3'). The 3' end of the gene was cleaved at position 4211 with the enzyme NdeI and blunt ended with the Klenow fragment of DNA polymerase.

The cloning vector pUC8 (Messing, J. and Vieira, J. [1982] Gene 19:269-276) which can be purchased from Pharmacia, Piscataway, NJ, was cleaved with SalI and EcoRI and cloned into plasmid pBR322 which had been cut with the same enzymes. The trp promoter (Genblock, available from Pharmacia) was blunt ended at the 5' end with Klenow and inserted into this hybrid vector by blunt end ligation of the 5' end to the SmaI site of the vector, and by insertion of the 3' end at the SalI site of the vector. The k-1 gene was then inserted using the SalI site at the 5' end and by blunt end ligation of the 3' end to the PvuII site of the vector. A schematic drawing of this construct, called pEW1, is shown in Fig. 1 of the drawings.

Plasmid pEW1 contains the DNA sequence encoding Bacillus thuringiensis toxin k-1.

Example 2--Construction of plasmid pEW2

The k-73 gene is the HD-73 gene described by Adang et al. (Gene 36:289-300 1985). The k-73 gene was cleaved at position 176 with NsiI. The sequence was then cleaved at position 3212 with HindIII and the 3036 base fragment consisting of residues 176-3212 was isolated by agarose gel electrophoresis.

Plasmid pEW1, prepared as described in Example 1, was also cleaved with HindIII (position 3345 in Table 1) and partially digested with NsiI (position 556 in Table 1). The 3036 base fragment from k-73,

disclosed above, was inserted into the NsiI to HindIII region of pEW1 replacing the comparable fragment of the k-1 gene, and creating plasmid pEW2. A schematic diagram of pEW2 is shown in Fig. 2 of the drawings.

Plasmid pEW2 contains the DNA sequence encoding Bacillus thuringiensis toxin k-73.

Example 3--Construction of plasmid pEW3

The k-1 gene was cut with SacI at position 1873. The gene was then submitted to partial digestion with HindIII and the 1427 base fragment consisting of residues 1873 to 3345 was isolated by agarose gel electrophoresis. Plasmid pEW2 was cut with SacI and HindIII and the large fragment representing the entire plasmid minus the SacI to HindIII fragment of the k-2 gene was isolated by agarose gel electrophoresis. The 1427 base fragment from the k-1 gene was then ligated into the SacI to HindIII region of pEW2, creating plasmid pEW3. A schematic diagram of pEW3 is shown in Fig. 3 of the drawings.

Plasmid pEW3 contains the DNA sequence encoding Bacillus thuringiensis chimeric toxin k-73/k-1 (pHY).

The nucleotide sequence encoding the chimeric toxin is shown in Table 1. The deduced amino acid sequence is shown in Table 1A.

Example 4--Construction of plasmid pEW4

The k-1 gene was cut at position 556 with NsiI. The gene was then cut with SacI at position 1873 and the 1317 base fragment from NsiI to SacI was isolated by agarose gel electrophoresis. Plasmid pEW2 was cut with SacI and then submitted to partial digestion with NsiI. The large fragment representing the entire

plasmid, minus the NsiI to SacI region of the k-73 gene, was isolated by agarose gel electrophoresis. The 1317 base NsiI to SacI fragment of gene k-1 was then ligated into NsiI to SacI region of pEW2 to create plasmid pEW4. A schematic diagram of pEW4 is shown in Fig. 4 of the drawings.

The nucleotide sequence encoding the chimeric toxin is shown in Table 2. The deduced amino acid sequence is shown in Table 2A.

Plasmid pEW4 contains the DNA sequence encoding Bacillus thuringiensis chimeric toxin k-1/k-73 (PYH).

Example 5--Insertion of Chimeric Toxin Genes Into Plants

Genes coding for chimeric insecticidal toxins, as disclosed herein, can be inserted into plant cells using the Ti plasmid from Agrobacter tumefaciens. Plant cells can then be caused to regenerate into plants (Zambryski, P., Joos, H., Gentello, C., Leemans, J., Van Montague, M. and Schell, J. [1983] EMBO J. 2:2143-2150; Bartok, K., Binns, A., Matzke, A. and Chilton, M-D. [1983] Cell 32:1033-1043). A particularly useful vector in this regard is pEND4K (Klee, H.J., Yanofsky, M.F. and Nester, E.W. [1985] Bio/Technology 3:637-642). This plasmid can replicate both in plant cells and in bacteria and has multiple cloning sites for passenger genes. Toxin genes, for example, can be inserted into the BamHI site of pEND4K, propagated in E. coli, and transformed into appropriate plant cells.

Example 6--Cloning of B. thuringiensis genes into baculoviruses

Genes coding for Bacillus thuringiensis chimeric toxins, as disclosed herein, can be cloned

into baculoviruses such as Autographa californica nuclear polyhedrosis virus (AcNPV). Plasmids can be constructed that contain the AcNPV genome cloned into a commercial cloning vector such as pUC8. The AcNPV genome is modified so that the coding region of the polyhedrin gene is removed and a unique cloning site for a passenger gene is placed directly behind the polyhedrin promoter. Examples of such vectors are pGP-B6874, described by Pennock et al. (Pennock, G.D., Shoemaker, C. and Miller, L.K. [1984] Mol. Cell. Biol. 4:399-406), and pAC380, described by Smith et al. (Smith, G.E., Summers, M.D. and Fraser, M.J. [1983] Mol. Cell. Biol. 3:2156-2165). The genes coding for k-1, k-73, k-73/k-1, k-1/k-73, or other B.t. genes can be modified with BamHI linkers at appropriate regions both upstream and downstream from the coding regions and inserted into the passenger site of one of the AcNPV vectors.

Example 7--Chimeric Toxin Denoted ACB-1

Enhanced toxicity against all three insects tested was shown by a toxin denoted ACB-1. The toxin ACB-1 (Table 3A) is encoded by plasmid pACB-1 (Table 3). The insecticidal activity encoded by pACB-1, in comparison with pEW3 (Example 3), is as follows:

	LC_{50} (O.D.$_{575}$/ml)		
Clone	T. ni	H. zea	S. exigua
pEW3	4.3	23.0	12.3
pACB-1	1.2	3.9	1.2

The above test was conducted using the conditions described previously.

The above results show that the ACB-1 toxin has the best composite activity as compared to the other toxins tested herein against all three insects.

Plasmid pACB-1 was constructed between the variable region of MTX-36, a wild B. thuringiensis strain, having the deposit accession number NRRL B-18101, and the variable region of HD-73 as follows: MTX-36; N-terminal to SacI site. HD-73; SacI site to C-terminal.

Total plasmid DNA was prepared from strain MTX-36 by standard procedures. The DNA was submitted to complete digestion by restriction enzymes SpeI and DraI. The digest was separated according to size by agarose gel electrophoresis and a 1962 bp fragment was purified by electroelution using standard procedures.

Plasmid pEW2 was purified and digested completely with SpeI and then submitted to partial digestion with DraI. The digest was submitted to agarose gel electrophoresis and a 4,138 bp fragment was purified by electroelution as above.

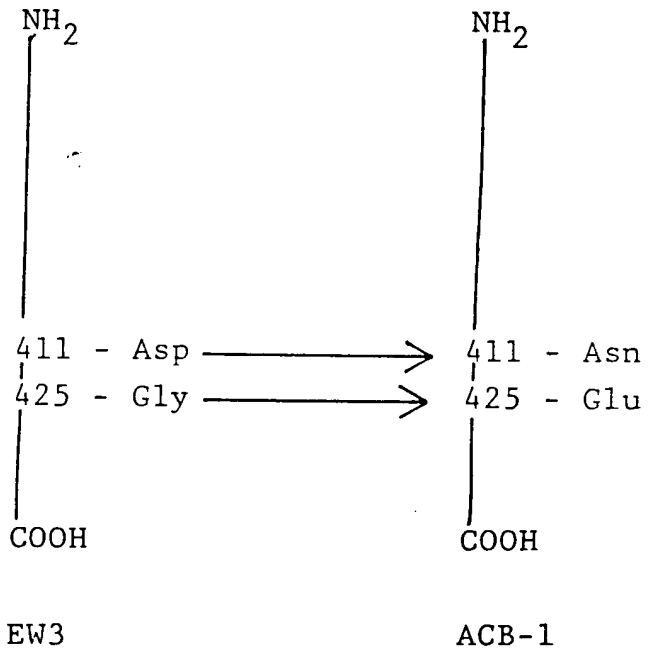
The two fragments (1962 bp from MTX-36 and 4138 bp from pEW2 were ligated together to form construct pACB.

Plasmid DNA was prepared from pACB, digested completely with SacI and NdeI and a 3760 bp fragment was isolated by electroelution following agarose gel electrophoresis.

Plasmid pEW1 was digested completely with SacI and NdeI and a 2340 bp fragment was isolated by electroelution following agarose gel electrophoresis.

The two fragments (3760 bp from pACB and 2340 from pEW1) were ligated together to form construct pACB-1.

The complete nucleotide sequence of the ACB-1 gene was determined and the deduced amino acid sequence of the toxin was compared with that determined for the toxin encoded by pEW3 (EW3). The result was that the deduced amino acid sequence of the ACB-1 toxin was identical to that of EW3 with two exceptions: (1) Aspartic acid residue 411 in EW3 was changed to asparagine in ACB-1 and (2) glycine residue 425 in EW3 was changed to glutamic acid in ACB-1. These two amino acid changes account for all of the changes in insect toxicity between these strains. The amino acid sequence of the EW3 toxin is as reported in Table 1. A schematic representation of these two toxins is as follows:



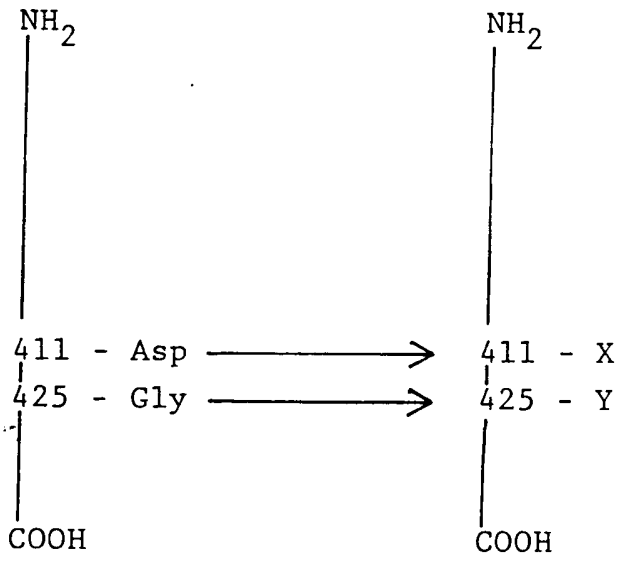
The above disclosure is further exemplification of the subject invention process for altering the host range of Bacillus toxins which comprises recombining in vitro the variable region of two or more toxin genes. Once a chimeric toxin is produced, the gene encoding the same can be sequenced by standard procedures, as disclosed above. The sequencing data can be used to alter other DNA by known molecular biology procedures to obtain the desired novel toxin. For example, the above-noted changes in the ACB-1 gene from HD-73, makes it possible to construct the ACB-1 gene as follows:

Plasmid pEW3, NRRL B-18034, was modified by altering the coding sequence for the toxin. The 151 bp DNA fragment bounded by the AccI restriction site at nucleotide residue 1199 in the coding sequence, and the SacI restriction site at residue 1350 were removed by digestion with the indicated restriction endonucleases using standard procedures. The removed 151 bp DNA fragment was replaced with the following synthetic DNA oligomer by standard procedures:

A TAC AGA AAA AGC GGA ACG GTA GAT TCG CTG AAT GAA
ATA CCG CCA CAG AAT AAC AAC GTG CCC CCG AGG CAA
GAA TTT AGT CAT CGA TTA AGC CAT GTT TCA ATG TTT
AGA TCT GGC TTT AGT AAT AGT AGT GTA AGT ATA ATA
AGA GCT

The net result of this change is that the aspartic residue at position 411 in the toxin encoded by pEW3 (Table 1A) is converted to asparagine, and the glycine residue at position 425 is converted to a glutamic residue. All other amino acids encoded by these genes are identical.

The changes made at positions 411 and 425, discussed above, clearly illustrate the sensitivity of these two positions in toxin EW3. Accordingly, the scope of the invention is not limited to the particular amino acids depicted as participating in the changes. The scope of the invention includes substitution of all 19 other amino acids at these positions. This can be shown by the following schematic:



EW3

wherein X is one of the 20 common amino acids except Asp when the amino acid at position 425 is Gly; Y is one of the 20 common amino acids except Gly when the amino acid at position 411 is Asp. The 20 common amino acids are as follows: alanine, arginine, asparagine, aspartate, cysteine, glutamine, glutamate, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine.

Example 8--Chimeric Toxin Denoted SYW1

Enhanced toxicity against tested insects was shown by a toxin denoted SYW1. The toxin SYW1 (Table 4A) is encoded by plasmid pSYW1 (Table 4). The insecticidal activity encoded by pSYW1, in comparison with pEW1 (Example 1) and pEW2 (Example 2), is as follows:

	LC_{50} (O.D.$_{575}$/ml)		
Clone	T. ni	H. zea	S. exigua
pEW1	3.5	12.3	18.8
pEW2	1.4	52.3	5.9
pSYW1	0.7	1.9	12.0

The above test was conducted using the conditions described previously.

Plasmid pSYW1 was constructed as follows:

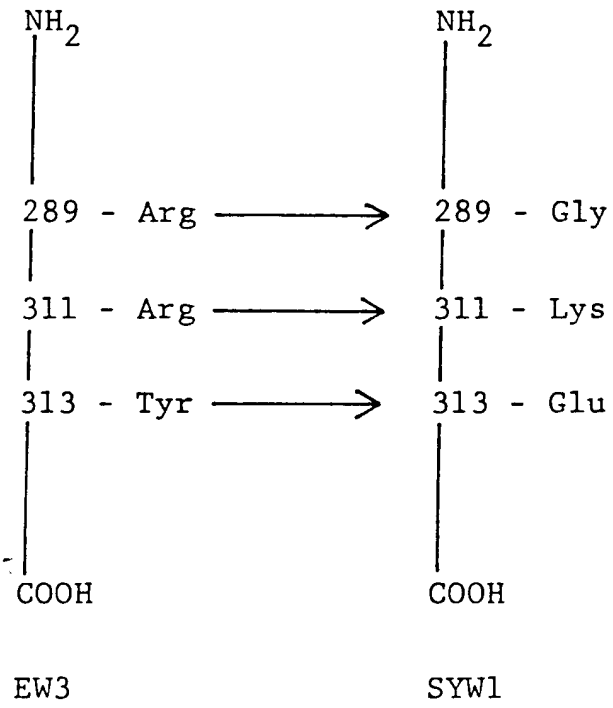
Plasmid DNA from pEW2 was prepared by standard procedures and submitted to complete digestion with restriction enzyme AsuII followed by partial digestion with EcoRI. A 5878 bp fragment was purified by electroelution following agarose gel electrophoresis of the digest by standard procedures.

Plasmid DNA from strain HD-1 was prepared and submitted to complete digestion with restriction enzymes AsuII and EcoRI. A 222 bp fragment was purified by electroelution following agarose gel electrophoresis of the digest.

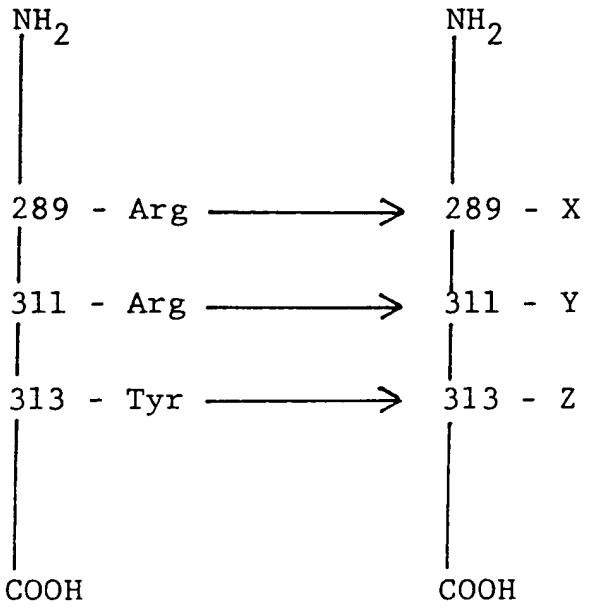
The two fragments (5878 bp from pEW2 and 222 bp from HD-1) were ligated together, by standard procedures, to form construct pSYW1.

The amino acid changes (3) in toxin SYW1 from EW3 are as follows: (1) Arginine residue 289 in EW3 was

changed to glycine in SYW1, (2) arginine residue 311 in EW3 was changed to lysine in SYW1, and (3) the tyrosine residue 313 was changed to glycine in SYW1. A schematic representation of these two toxins is as follows:



The changes made at positions 289, 311, and 313, discussed above, clearly illustrate the sensitivity of these three positions in toxin EW3. Accordingly, the scope of the invention is not limited to the particular amino acids depicted as participating in the changes. The scope of the invention includes substitution of all the common amino acids at these positions. This can be shown by the following schematic:



EW3

wherein X is one of the 20 common amino acids except Arg when the amino acid at position 311 is Arg and the amino acid at position 313 is Tyr; Y is one of the 20 common amino acids except Arg when the amino acid at position 289 is Arg and the amino acid at position 313 is Tyr; and Z is one of the 20 common amino acids except Tyr when the amino acid at position 289 is Arg and the amino acid at position 311 is Arg.

Construction of the SYW1 gene can be carried out by procedures disclosed above for the construction of the ACB-1 gene from plasmid pEW3 with appropriate changes in the synthetic DNA oligomer.

As is well known in the art, the amino acid sequence of a protein is determined by the nucleotide sequence of the DNA. Because of the redundancy of the genetic code, i.e., more than one coding nucleotide triplet (codon) can be used for most of the amino acids used to make proteins, different nucleotide sequences can code for a particular amino acid. Thus, the genetic code can be depicted as follows:

Phenylalanine (Phe)	TTK	Histidine (His)	CAK
Leucine (Leu)	XTY	Glutamine (Gln)	CAJ
Isoleucine (Ile)	ATM	Asparagine (Asn)	AAK
Methionine (Met)	ATG	Lysine (Lys)	AAJ
Valine (Val)	GTL	Aspartic acid (Asp)	GAK
Serine (Ser)	QRS	Glutamic acid (Glu)	GAJ
Proline (Pro)	CCL	Cysteine (Cys)	TGK
Threonine (Thr)	ACL	Tryptophan (Trp)	TGG
Alanine (Ala)	GCL	Arginine (Arg)	WGZ
Tyrosine (Tyr)	TAK	Glycine (Gly)	GGL
Termination signal	TAJ		

Key: Each 3-letter deoxynucleotide triplet corresponds to a trinucleotide of mRNA, having a 5'-end on the left and a 3'-end on the right. All DNA sequences given herein are those of the strand whose sequence corresponds to the mRNA sequence, with thymine substituted for uracil. The letters stand for the purine or pyrimidine bases forming the deoxynucleotide sequence.

A = adenine
G - guanine
C = cytosine
T = thymine
X = T or C if Y is A or G
X = C if Y is C or T
Y = A, G, C or T if X is C
Y = A or G if X is T

W = C or A if Z is A or G
W = C if Z is C or T
Z = A, G, C or T if W is C
Z = A or G if W is A
QR = TC if S is A, G, C or T; alternatively QR = AG if S is T or C
J = A or G
K = T or C
L = A, T, C or G
M = A, C or T

The above shows that the novel amino acid sequence of the chimeric toxins, and other useful proteins, can be prepared by equivalent nucleotide sequences encoding the same amino acid sequence of the proteins. Accordingly, the subject invention includes such equivalent nucleotide sequences. In addition it has been shown that proteins of identified structure and function may be constructed by changing the amino acid sequence if such changes do not alter the protein secondary structure (Kaiser, E.T. and Kezdy, F.J. [1984] Science 223:249-255). Thus, the subject invention includes muteins of the amino acid sequences depicted herein which do not alter the protein secondary structure.

The one-letter symbol for the amino acids used in Tables 1A and 2A is well known in the art. For convenience, the relationship of the three-letter abbreviation and the one-letter symbol for amino acids is as follows:

Ala	A
Arg	R
Asn	N
Asp	D

Cys	C
Gln	Q
Glu	E
Gly	G
His	H
Ile	I
Leu	L
Lys	K
Met	M
Phe	F
Pro	P
Ser	S
Thr	T
Trp	W
Tyr	Y
Val	V

The work described herein was all done in conformity with physical and biological containment requirements specified in the NIH Guidelines.

CHART A

Bioassay of Chimeric Toxins Against Various Insects

Plasmid	Toxin	LC50 (O.D. 575/ml diet)		
		T. ni	S. exigua	H. zea
pEW1	k-1	3.5	12.3	18.8
pEW2	k-73	1.4	52.3	5.9
pEW3	k-73/k-1	5.7	9.6	10.4
pEW4	k-1/k-73	0.8	30.4	2.2

Recombinant E. coli cells containing the above plasmids were grown overnight in L-broth.* The cells were pelleted and resuspended on 0.85% NaCl. The optical density at 575 nm was determined for these cell suspensions and appropriate dilutions were made in 0.85% NaCl. Three ml of each dilution were added to 27 ml of USDA diet (Dulmage, H.D., Martinez, A.J. and Pena, T [1976] USDA Agricultural Research Service Technical Bulletin No. 1528, U.S. Government Printing Office, Washington, D.C.). The diet/toxin mixture was then dispensed into 24 wells in a plastic tissue culture tray (1.0 ml/well). Single neonate larvae from either Trichoplusia ni, Spodoptera exigua, or Heliothis zea were then added to each well. The trays were then covered with Mylar and punctured with small holes for air exchange. The larvae were observed after 7 days and LC50 values were calculated using the method of probit analysis (Finney, D.J. [1971] Probit Analysis 3rd ed. Cambridge University Press, Cambridge).

*L-broth is 5 g/l NaCl, 10 g/l bactotryptone, 5 g/l yeast extract.

CHART B

Assay of Toxins Against CF-1 Cells in Culture

Plasmid	Toxin	Live Cells (% of Control)	
		Expt. 1	Expt. 2
pEW1	k-1	106%	108%
pEW2	k-73	44%	46%
pEW3	k-73/k-1	105%	97%
pEW4	k-1/k-73	53%	58%

Overnight cultures of E. coli containing the various plasmids were centrifuged and resuspended in 0.85% NaCl containing 1 mM EDTA[1], 0.2 mM PMSF[2], 0.2 mM TPCK[3] and 100 mM NaOH. Cells were broken in a bead beater (Biospec Products, Bartlesville, OK), centrifuged and the supernatant dialyzed against 20 mM Tris-glycine pH 8.5. Toxin was activated with 0.7% trypsin. Assays were carried out on Choristoneura fumiferana cell line CF-1. Approximately 100 µg of activated toxin extract was added to 3.2×10^5 cells in a volume of 1.0 ml. ATP levels were determined after 30 min incubation and the percentage of live cells remaining in the suspension was determined from standard curves.

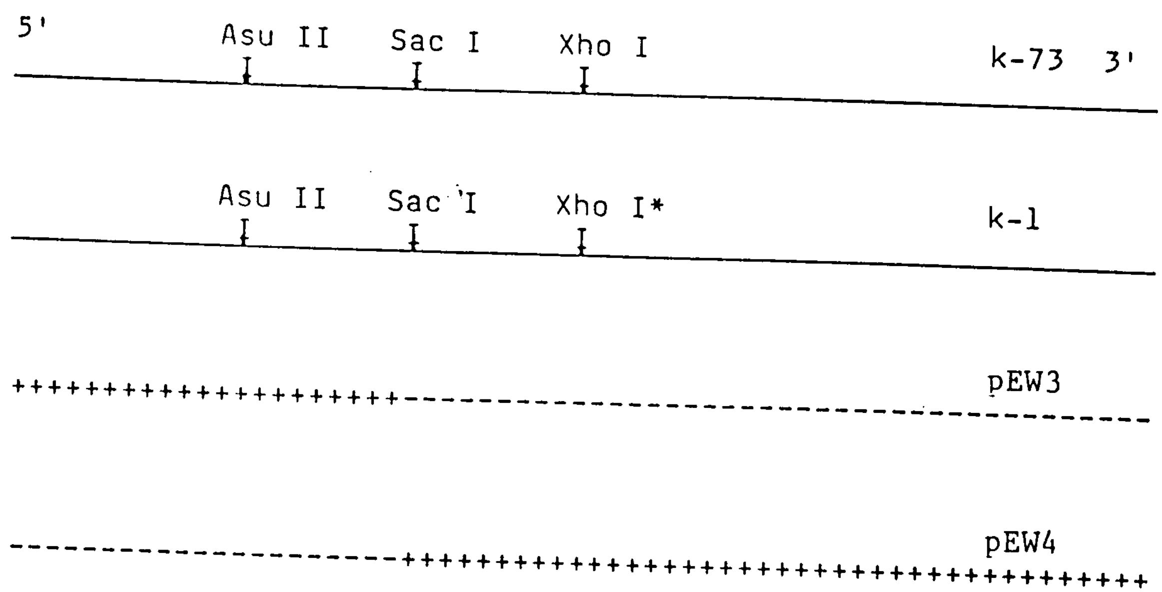
[1] ethylenediaminetetraacetic acid

[2] phenylmethylsulfonyl fluoride

[3] 1-tosylamide-2-phenylethylchloromethyl ketone

CHART C

Facile Comparison of Constructions of Plasmids pEW3 and pEW4



---- = sequences from k-1
++++ = sequences from k-73
Xho I* means that this restriction site found in k-73 no longer exists in k-1 and will have to be recreated by site specific mutagenesis (it involves changing two base pairs in k-1).

Table 1

Nucleotide Sequence of Plasmid pEW3 Encoding Chimeric Toxin

Numbering of the nucleotide bases is the same as Schnepf et al. (J. Biol. Chem. 260:6264-6272 [1985]) for HD-1 and Adang et al. (Gene 36:289-300 [1985]) for HD-73. Only protein coding sequences are shown.

```
                (start HD-73)                ATG GATAACAATC 400
CGAACATCAA TGAATGCATT CCTTATAATT GTTTAAGTAA CCCTGAAGTA
GAAGTATTAG GTGGAGAAAG AATAGAAACT GGTTACACCC CAATCGATAT 500
TTCCTTGTCG CTAACGCAAT TTCTTTTGAG TGAATTTGTT CCCGGTGCTG
GATTTGTGTT AGGACTAGTT GATATAATAT GGGGAATTTT TGGTCCCTCT 600
CAATGGGACG CATTTCTTGT ACAAATTGAA CAGTTAATTA ACCAAAGAAT
AGAAGAATTC GCTAGGAACC AAGCCATTTC TAGATTAGAA GGACTAAGCA 700
ATCTTTATCA AATTTACGCA GAATCTTTTA GAGAGTGGGA AGCAGATCCT
ACTAATCCAG CATTAAGAGA AGAGATGCGT ATTCAATTCA ATGACATGAA 800
CAGTGCCCTT ACAACCGCTA TTCCTCTTTT TGCAGTTCAA AATTATCAAG
TTCCTCTTTT ATCAGTATAT GTTCAAGCTG CAAATTTACA TTTATCAGTT 900
TTGAGAGATG TTTCAGTGTT TGGACAAAGG TGGGGATTTG ATGCCGCGAC
TATCAATAGT CGTTATAATG ATTTAACTAG GCTTATTGGC AACTATACAG 1000
ATTATGCTGT ACGCTGGTAC AATACGGGAT TAGAACGTGT ATGGGGACCG
GATTCTAGAG ATTGGGTAAG GTATAATCAA TTTAGAAGAG AATTAACACT 1100
AACTGTATTA GATATCGTTG CTCTGTTCCC GAATTATGAT AGTAGAAGAT
ATCCAATTCG AACAGTTTCC CAATTAACAA GAGAAATTTA TACAAACCCA 1200
GTATTAGAAA ATTTTGATGG TAGTTTTCGA GGCTCGGCTC AGGGCATAGA
AAGAAGTATT AGGAGTCCAC ATTTGATGGA TATACTTAAC AGTATAACCA 1300
TCTATACGGA TGCTCATAGG GGTTATTATT ATTGGTCAGG GCATCAAATA
ATGGCTTCTC CTGTAGGGTT TTCGGGGCCA GAATTCACTT TTCCGCTATA 1400
TGGAACTATG GGAAATGCAG CTCCACAACA ACGTATTGTT GCTCAACTAG
GTCAGGGCGT GTATAGAACA TTATCGTCCA CTTTATATAG AAGACCTTTT 1500
AATATAGGGA TAAATAATCA ACAACTATCT GTTCTTGACG GGACAGAATT
TGCTTATGGA ACCTCCTCAA ATTTGCCATC CGCTGTATAC AGAAAAAGCG 1600
GAACGGTAGA TTCGCTGGAT GAAATACCGC CACAGAATAA CAACGTGCCA
CCTAGGCAAG GATTTAGTCA TCGATTAAGC CATGTTTCAA TGTTTCGTTC 1700
AGGCTTTAGT AATAGTAGTG TAAGTATAAT AAGAGCT (end hd-73)
    (start HD-1)          CCAACGT TTTCTTGGCA GCATCGCAGT 1900
GCTGAATTTA ATAATATAAT TCCTTCATCA CAAATTACAC AAATACCTTT
AACAAAATCT ACTAATCTTG GCTCTGGAAC TTCTGTCGTT AAAGGACCAG 2000
GATTTACAGG AGGAGATATT CTTCGAAGAA CTTCACCTGG CCAGATTTCA
ACCTTAAGAG TAAATATTAC TGCACCATTA TCACAAAGAT ATCGGGTAAG 2100
AATTCGCTAC GCTTCTACTA CAAATTTACA ATTCCATACA TCAATTGACG
GAAGACCTAT TAATCAGGGT AATTTTTCAG CAACTATGAG TAGTGGGAGT 2200
AATTTACAGT CCGGAAGCTT TAGGACTGTA GGTTTTACTA CTCCGTTTAA
CTTTTCAAAT GGATCAAGTG TATTTACGTT AAGTGCTCAT GTCTTCAATT 2300
CAGGCAATGA AGTTTATATA GATCGAATTG AATTTGTTCC GGCAGAAGTA
ACCTTTGAGG CAGAATATGA TTTAGAAAGA GCACAAAAGG CGGTGAATGA 2400
GCTGTTTACT TCTTCCAATC AAATCGGGTT AAAAACAGAT GTGACGGATT
```

Table 1 (cont.)

ATCATATTGA TCAAGTATCC AATTTAGTTG AGTGTTTATC AGATGAATTT 2500
TGTCTGGATG AAAAACAAGA ATTGTCCGAG AAAGTCAAAC ATGCGAAGCG
ACTTAGTGAT GAGCGGAATT TACTTCAAGA TCCAAACTTC AGAGGGATCA 2600
ATAGACAACT AGACCGTGGC TGGAGAGGAA GTACGGATAT TACCATCCAA
GGAGGCGATG ACGTATTCAA AGAGAATTAC GTTACGCTAT TGGGTACCTT 2700
TGATGAGTGC TATCCAACGT ATTTATATCA AAAAATAGAT GAGTCGAAAT
TAAAAGCCTA TACCCGTTAT CAATTAAGAG GGTATATCGA AGATAGTCAA 2800
GACTTAGAAA TCTATTTAAT TCGCTACAAT GCAAAACATG AAACAGTAAA
TGTGCCAGGT ACGGGTTCCT TATGGCCGCT TTCAGCCCAA AGTCCAATCG 2900
GAAAGTGTGG AGAGCCGAAT CGATGCGCGC CACACCTTGA ATGGAATCCT
GACTTAGATT GTTCGTGTAG GGATGGAGAA AAGTGTGCCC ATCATTCGCA 3000
TCATTTCTCC TTAGACATTG ATGTAGGATG TACAGACTTA AATGAGGACC
TAGGTGTATG GGTGATCTTT AAGATTAAGA CGCAAGATGG GCACGCAAGA 3100
CTAGGGAATC TAGAGTTTCT CGAAGAGAAA CCATTAGTAG GAGAAGCGCT
AGCTCGTGTG AAAAGAGCGG AGAAAAAATG GAGAGACAAA CGTGAAAAAT 3200
TGGAATGGGA AACAAATATC GTTTATAAAG AGGCAAAAGA ATCTGTAGAT
GCTTTATTTG TAAACTCTCA ATATGATCAA TTACAAGCGG ATACGAATAT 3300
TGCCATGATT CATGCGGCAG ATAAACGTGT TCATAGCATT CGAGAAGCTT
ATCTGCCTGA GCTGTCTGTG ATTCCGGGTG TCAATGCGGC TATTTTTGAA 3400
GAATTAGAAG GGCGTATTTT CACTGCATTC TCCCTATATG ATGCGAGAAA
TGTCATTAAA AATGGTGATT TTAATAATGG CTTATCCTGC TGGAACGTGA 3500
AAGGGCATGT AGATGTAGAA GAACAAAACA ACCAACGTTC GGTCCTTGTT
CTTCCGGAAT GGGAAGCAGA AGTGTCACAA GAAGTTCGTG TCTGTCCGGG 3600
TCGTGGCTAT ATCCTTCGTG TCACAGCGTA CAAGGAGGGA TATGGAGAAG
GTTGCGTAAC CATTCATGAG ATCGAGAACA ATACAGACGA ACTGAAGTTT 3700
AGCAACTGCG TAGAAGAGGA AATCTATCCA AATAACACGG TAACGTGTAA
TGATTATACT GTAAATCAAG AAGAATACGG AGGTGCGTAC ACTTCTCGTA 3800
ATCGAGGATA TAACGAAGCT CCTTCCGTAC CAGCTGATTA TGCGTCAGTC
TATGAAGAAA AATCGTATAC AGATGGACGA AGAGAGAATC CTTGTGAATT 3900
TAACAGAGGG TATAGGGATT ACACGCCACT ACCAGTTGGT TATGTGACAA
AAGAATTAGA ATACTTCCCA GAAACCGATA AGGTATGGAT TGAGATTGGA 4000
GAAACGGAAG GAACATTTAT CGTGGACAGC GTGGAATTAC TCCTTATGGA
GGAA (end HD-1)

Table 1A

Deduced Amino Acid Sequence of Chimeric Toxin Produced by Plasmid pEW3

```
M D N N P N I N E C I P Y N C L S N P E V E V L G G E R I E
T G Y T P I D I S L S L T Q F L L S E F V P G A G F V L G L
V D I I W G I F G P S Q W D A F L V Q I E Q L I N Q R I E E
F A R N Q A I S R L E G L S N L Y Q I Y A E S F R E W E A D
P T N P A L R E E M R I Q F N D M N S A L T T A I P L F A V
Q N Y Q V P L L S V Y V Q A A N L H L S V L R D V S V F G Q
R W G F D A A T I N S R Y N D L T R L I G N Y T D Y A V R W
Y N T G L E R V W G P D S R D W V R Y N Q F R R E L T L T V
L D I V A L F P N Y D S R R Y P I R T V S Q L T R E I Y T N
P V L E N F D G S F R G S A Q G I E R S I R S P H L M D I L
N S I T I Y T D A H R G Y Y Y W S G H Q I M A S P V G F S G
P E F T F P L Y G T M G N A A P Q Q R I V A Q L G Q G V Y R
T L S S T L Y R R P F N I G I N N Q Q L S V L D G T E F A Y
G T S S N L P S A V Y R K S G T V D S L D E I P P Q N N N V
P P R Q G F S H R L S H V S M F R S G F S N S S V S I I R A
P T F S W Q H R S A E F N N I I P S S Q I T Q I P L T K S T
N L G S G T S V V K G P G F T G G D I L R R T S P G Q I S T
L R V N I T A P L S Q R Y R V R I R Y A S T T N L Q F H T S
I D G R P I N Q G N F S A T M S S G S N L Q S G S F R T V G
F T T P F N F S N G S S V F T L S A H V F N S G N E V Y I D
R I E F V P A E V T F E A E Y D L E R A Q K A V N E L F T S
S N Q I G L K T D V T D Y H I D Q V S N L V E C L S D E F C
L D E K Q E L S E K V K H A K R L S D E R N L L Q D P N F R
G I N R Q L D R G W R G S T D I T I Q G G D D V F K E N Y V
T L L G T F D E C Y P T Y L Y Q K I D E S K L K A Y T R Y Q
L R G Y I E D S Q D L E I Y L I R Y N A K H E T V N V P G T
G S L W P L S A Q S P I G K C G E P N R C A P H L E W N P D
L D C S C R D G E K C A H H S H H F S L D I D V G C T D L N
E D L G V W V I F K I K T Q D G H A R L G N L E F L E E K P
L V G E A L A R V K R A E K K W R D K R E K L E W E T N I V
Y K E A K E S V D A L F V N S Q Y D Q L Q A D T N I A M I H
A A D K R V H S I R E A Y L P E L S V I P G V N A A I F E E
L E G R I F T A F S L Y D A R N V I K N G D F N N G L S C W
N V K G H V D V E E Q N N Q R S V L V L P E W E A E V S Q E
V R V C P G R G Y I L R V T A Y K E G Y G E G C V T I H E I
E N N T D E L K F S N C V E E E I Y P N N T V T C N D Y T V
N Q E E Y G G A Y T S R N R G Y N E A P S V P A D Y A S V Y
E E K S Y T D G R R E N P C E F N R G Y R D Y T P L P V G Y
V T K E L E Y F P E T D K V W I E I G E T E G T F I V D S V
E L L L M E E
```

Table 2

Nucleotide Sequence of Plasmid pEW4 Encoding Chimeric Toxin

Numbering of nucleotide bases is the same as Schnepf et al. (J. Biol. Chem. 260:6264-6272 [1985]) for HD-1 and Adang et al. (Gene 36:289-300 [1985]) for HD-73. Only protein coding sequences are shown.

```
      (start HD-1)          ATGG ATAACAATCC GAACATCAAT
GAATGCATTC CTTATAATTG TTTAAGTAAC CCTGAAGTAG AAGTATTAGG 600
TGGAGAAAGA ATAGAAACTG GTTACACCCC AATCGATATT TCCTTGTCGC
TAACGCAATT TCTTTTGAGT GAATTTGTTC CCGGTGCTGG ATTTGTGTTA 700
GGACTAGTTG ATATAATATG GGGAATTTTT GGTCCCTCTC AATGGGACGC
ATTTCCTGTA CAAATTGAAC AGTTAATTAA CCAAAGAATA GAAGAATTCG 800
CTAGGAACCA AGCCATTTCT AGATTAGAAG GACTAAGCAA TCTTTATCAA
ATTTACGCAG AATCTTTTAG AGAGTGGGAA GCAGATCCTA CTAATCCAGC 900
ATTAAGAGAA GAGATGCGTA TTCAATTCAA TGACATGAAC AGTGCCCTTA
CAACCGCTAT TCCTCTTTTG GCAGTTCAAA ATTATCAAGT TCCTCTTTTA 1000
TCAGTATATG TTCAAGCTGC AAATTTACAT TTATCAGTTT TGAGAGATGT
TTCAGTGTTT GGACAAAGGT GGGGATTTGA TGCCGCGACT ATCAATAGTC 1100
GTTATAATGA TTTAACTAGG CTTATTGGCA ACTATACAGA TTATGCTGTG
CGCTGGTACA ATACGGGATT AGAGCGTGTA TGGGGACCGG ATTCTAGAGA 1200
TTGGGTAAGG TATAATCAAT TTAGAAGAGA GCTAACACTT ACTGTATTAG
ATATCGTTGC TCTATTCTCA AATTATGATA GTCGAAGGTA TCCAATTCGA 1300
ACAGTTTCCC AATTAACAAG AGAAATTTAT ACGAACCCAG TATTAGAAAA
TTTTGATGGT AGTTTTCGTG GAATGGCTCA GAGAATAGAA CAGAATATTA 1400
GGCAACCACA TCTTATGGAT ATCCTTAATA GTATAACCAT TTATACTGAT
GTGCATAGAG GCTTTAATTA TTGGTCAGGG CATCAAATAA CAGCTTCTCC 1500
TGTAGGGTTT TCAGGACCAG AATTCGCATT CCCTTTATTT GGGAATGCGG
GGAATGCAGC TCCACCCGTA CTTGTCTCAT TAACTGGTTT GGGGATTTTT 1600
AGAACATTAT CTTCACCTTT ATATAGAAGA ATTATACTTG GTTCAGGCCC
AAATAATCAG GAACTGTTTG TCCTTGATGG AACGGAGTTT TCTTTTGCCT 1700
CCCTAACGAC CAACTTGCCT TCCACTATAT ATAGACAAAG GGGTACAGTC
GATTCACTAG ATGTAATACC GCCACAGGAT AATAGTGTAC CACCTCGTGC 1800
GGGATTTAGC CATCGATTGA GTCATGTTAC AATGCTGAGC CAAGCAGCTG
GAGCAGTTTA CACCTTGAGA GCTCAACGT  (stop HD-1)
            (start HD-73)              CCT ATGTTCTCTT
GGATACATCG TAGTGCTGAA TTTAATAATA TAATTGCATC GGATAGTATT 1800
ACTCAAATCC CTGCAGTGAA GGGAAACTTT CTTTTTAATG GTTCTGTAAT
TTCAGGACCA GGATTTACTG GTGGGGACTT AGTTAGATTA AATAGTAGTG 1900
GAAATAACAT TCAGAATAGA GGGTATATTG AAGTTCCAAT TCACTTCCCA
TCGACATCTA CCAGATATCG AGTTCGTGTA CGGTATGCTT CTGTAACCCC 2000
GATTCACCTC AACGTTAATT GGGGTAATTC ATCCATTTTT TCCAATACAG
TACCAGCTAC AGCTACGTCA TTAGATAATC TACAATCAAG TGATTTTGGT 2100
TATTTTGAAA GTGCCAATGC TTTTACATCT TCATTAGGTA ATATAGTAGG
TGTTAGAAAT TTTAGTGGGA CTGCAGGAGT GATAATAGAC AGATTTGAAT 2200
TTATTCCAGT TACTGCAACA CTCGAGGCTG AATATAATCT GGAAAGAGCG
```

Table 2 (cont.)

CAGAAGGCGG TGAATGCGCT GTTTACGTCT ACAAACCAAC TAGGGCTAAA 2300
AACAAATGTA ACGGATTATC ATATTGATCA AGTGTCCAAT TTAGTTACGT
ATTTATCGGA TGAATTTTGT CTGGATGAAA AGCGAGAATT GTCCGAGAAA 2400
GTCAAACATG CGAAGCGACT CAGTGATGAA CGCAATTTAC TCCAAGATTC
AAATTTCAAA GACATTAATA GGCAACCAGA ACGTGGGTGG GGCGGAAGTA 2500
CAGGGATTAC CATCCAAGGA GGGGATGACG TATTTAAAGA AAATTACGTC
ACACTATCAG GTACCTTTGA TGAGTGCTAT CCAACATATT TGTATCAAAA 2600
AATCGATGAA TCAAAATTAA AAGCCTTTAC CCGTTATCAA TTAAGAGGGT
ATATCGAAGA TAGTCAAGAC TTAGAAATCT ATTTAATTCG CTACAATGCA 2700
AAACATGAAA CAGTAAATGT GCCAGGTACG GGTTCCTTAT GGCCGCTTTC
AGCCCAAAGT CCAATCGGAA AGTGTGGAGA GCCGAATCGA TGCGCGCCAC 2800
ACCTTGAATG GAATCCTGAC TTAGATTGTT CGTGTAGGGA TGGAGAAAAG
TGTGCCCATC ATTCGCATCA TTTCTCCTTA GACATTGATG TAGGATGTAC 2900
AGACTTAAAT GAGGACCTAG GTGTATGGGT GATCTTTAAG ATTAAGACGC
AAGATGGGCA CGCAAGACTA GGGAATCTAG AGTTTCTCGA AGAGAAACCA 3000
TTAGTAGGAG AAGCGCTAGC TCGTGTGAAA AGAGCGGAGA AAAAATGGAG
AGACAAACGT GAAAAATTGG AATGGGAAAC AAATATCGTT TATAAAGAGG 3100
CAAAAGAATC TGTAGATGCT TTATTTGTAA ACTCTCAATA TGATCAATTA
CAAGCGGATA CGAATATTGC CATGATTCAT GCGGCAGATA AACGTGTTCA 3200
TAGCATTCGA GAAGCTTATC TGCCTGAGCT GTCTGTGATT CCGGGTGTCA
ATGCGGCTAT TTTTGAAGAA TTAGAAGGGC GTATTTTCAC TGCATTCTCC 3300
CTATATGATG CGAGAAATGT CATTAAAAAT GGTGATTTTA ATAATGGCTT
ATCCTGCTGG AACGTGAAAG GGCATGTAGA TGTAGAAGAA CAAAACAACC 3400
AACGTTCGGT CCTTGTTGTT CCGGAATGGG AAGCAGAAGT GTCACAAGAA
GTTCGTGTCT GTCCGGGTCG TGGCTATATC CTTCGTGTCA CAGCGTACAA 3500
GGAGGGATAT GGAGAAGGTT GCGTAACCAT TCATGAGATC GAGAACAATA
CAGACGAACT GAAGTTTAGC AACTGCGTAG AAGAGGAAAT CTATCCAAAT 3600
AACACGGTAA CGTGTAATGA TTATACTGTA AATCAAGAAG AATACGGAGG
TGCGTACACT TCTCGTAATC GAGGATATAA CGAAGCTCCT TCCGTACCAG 3700
CTGATTATGC GTCAGTCTAT GAAGAAAAAT CGTATACAGA TGGACGAAGA
GAGAATCCTT GTGAATTTAA CAGAGGGTAT AGGGATTACA CGCCACTACC 3800
AGTTGGTTAT GTGACAAAAG AATTAGAATA CTTCCCAGAA ACCGATAAGG
TATGGATTGA GATTGGAGAA ACGGAAGGAA CATTTATCGT GGACAGCGTG 3900
GAATTACTCC TTATGGAGGA A (end HD-73)

Table 2A

Deduced Amino Acid Sequence of Chimeric Toxin Produced by Plasmid pEW4

```
M D N N P N I N E C I P Y N C L S N P E V E V L G G E R I E
T G Y T P I D I S L S L T Q F L L S E F V P G A G F V L G L
V D I I W G I F G P S Q W D A F P V Q I E Q L I N Q R I E E
F A R N Q A I S R L E G L S N L Y Q I Y A E S F R E W E A D
P T N P A L R E E M R I Q F N D M N S A L T T A I P L L A V
Q N Y Q V P L L S V Y V Q A A N L H L S V L R D V S V F G Q
R W G F D A A T I N S R Y N D L T R L I G N Y T D Y A V R W
Y N T G L E R V W G P D S R D W V R Y N Q F R R E L T L T V
L D I V A L F S N Y D S R R Y P I R T V S Q L T R E I Y T N
P V L E N F D G S F R G M A Q R I E Q N I R Q P H L M D I L
N S I T I Y T D V H R G F N Y W S G H Q I T A S P V G F S G
P E F A F P L F G N A G N A A P P V L V S L T G L G I F R T
L S S P L Y R R I I L G S G P N N Q E L F V L D G T E F S F
A S L T T N L P S T I Y R Q R G T V D S L D V I P P Q D N S
V P P R A G F S H R L S H V T M L S Q A A G A V Y T L R A Q
R P M F S W I H R S A E F N N I I A S D S I T Q I P A V K G
N F L F N G S V I S G P G F T G G D L V R L N S S G N N I Q
N R G Y I E V P I H F P S T S T R Y R V R V R Y A S V T P I
H L N V N W G N S S I F S N T V P A T A T S L D N L Q S S D
F G Y F E S A N A F T S S L G N I V G V R N F S G T A G V I
I D R F E F I P V T A T L E A E Y N L E R A Q K A V N A L F
T S T N Q L G L K T N V T D Y H I D Q V S N L V T Y L S D E
F C L D E K R E L S E K V K H A K R L S D E R N L L Q D S N
F K D I N R Q P E R G W G G S T G I T I Q G G D D V F K E N
Y V T L S G T F D E C Y P T Y L Y Q K I D E S K L K A F T R
Y Q L R G Y I E D S Q D L E I Y L I R Y N A K H E T V N V P
G T G S L W P L S A Q S P I G K C G E P N R C A P H L E W N
P D L D C S C R D G E K C A H H S H H F S L D I D V G C T D
L N E D L G V W V I F K I K T Q D G H A R L G N L E F L E E
K P L V G E A L A R V K R A E K K W R D K R E K L E W E T N
I V Y K E A K E S V D A L F V N S Q Y D Q L Q A D T N I A M
I H A A D K R V H S I R E A Y L P E L S V I P G V N A A I F
E E L E G R I F T A F S L Y D A R N V I K N G D F N N G L S
C W N V K G H V D V E E Q N N Q R S V L V V P E W E A E V S
Q E V R V C P G R G Y I L R V T A Y K E G Y G E G C V T I H
E I E N N T D E L K F S N C V E E E I Y P N N T V T C N D Y
T V N Q E E Y G G A Y T S R N R G Y N E A P S V P A D Y A S
V Y E E K S Y T D G R R E N P C E F N R G Y R D Y T P L P V
G Y V T K E L E Y F P E T D K V W I E I G E T E G T F I V D
S V E L L L M E E
```

Table 3

Nucleotide Sequence of Plasmid pACB-1 Encoding Chimeric Toxin ACB-1

The nucleotide differences as compared to the sequence shown in Table 1 are underlined at positions 1618 and 1661 and code for amino acid changes at positions 411 and 425 as shown in Table 3A.

```
                (start HD-73)              ATG GATAACAATC 400
CGAACATCAA TGAATGCATT CCTTATAATT GTTTAAGTAA CCCTGAAGTA
GAAGTATTAG GTGGAGAAAG AATAGAAACT GGTTACACCC CAATCGATAT 500
TTCCTTGTCG CTAACGCAAT TTCTTTTGAG TGAATTTGTT CCCGGTGCTG
GATTTGTGTT AGGACTAGTT GATATAATAT GGGGAATTTT TGGTCCCTCT 600
CAATGGGACG CATTTCTTGT ACAAATTGAA CAGTTAATTA ACCAAAGAAT
AGAAGAATTC GCTAGGAACC AAGCCATTTC TAGATTAGAA GGACTAAGCA 700
ATCTTTATCA AATTTACGCA GAATCTTTTA GAGAGTGGGA AGCAGATCCT
ACTAATCCAG CATTAAGAGA AGAGATGCGT ATTCAATTCA ATGACATGAA 800
CAGTGCCCTT ACAACCGCTA TTCCTCTTTT TGCAGTTCAA AATTATCAAG
TTCCTCTTTT ATCAGTATAT GTTCAAGCTG CAAATTTACA TTTATCAGTT 900
TTGAGAGATG TTTCAGTGTT TGGACAAAGG TGGGGATTTG ATGCCGCGAC
TATCAATAGT CGTTATAATG ATTTAACTAG GCTTATTGGC AACTATACAG 1000
ATTATGCTGT ACGCTGGTAC AATACGGGAT TAGAACGTGT ATGGGGACCG
GATTCTAGAG ATTGGGTAAG GTATAATCAA TTTAGAAGAG AATTAACACT 1100
AACTGTATTA GATATCGTTG CTCTGTTCCC GAATTATGAT AGTAGAAGAT
ATCCAATTCG AACAGTTTCC CAATTAACAA GAGAAATTTA TACAAACCCA 1200
GTATTAGAAA ATTTTGATGG TAGTTTTCGA GGCTCGGCTC AGGGCATAGA
AAGAAGTATT AGGAGTCCAC ATTTGATGGA TATACTTAAC AGTATAACCA 1300
TCTATACGGA TGCTCATAGG GGTTATTATT ATTGGTCAGG GCATCAAATA
ATGGCTTCTC CTGTAGGGTT TTCGGGGCCA GAATTCACTT TTCCGCTATA 1400
TGGAACTATG GGAAATGCAG CTCCACAACA ACGTATTGTT GCTCAACTAG
GTCAGGGCGT GTATAGAACA TTATCGTCCA CTTTATATAG AAGACCTTTT 1500
AATATAGGGA TAAATAATCA ACAACTATCT GTTCTTGACG GGACAGAATT
TGCTTATGGA ACCTCCTCAA ATTTGCCATC CGCTGTATAC AGAAAAAGCG 1600
GAACGGTAGA TTCGCTGAAT GAAATACCGC CACAGAATAA CAACGTGCCA
CCTAGGCAAG AATTTAGTCA TCGATTAAGC CATGTTTCAA TGTTTCGTTC 1700
AGGCTTTAGT AATAGTAGTG TAAGTATAAT AAGAGCT (end hd-73)
    (start HD-1)          CCAACGT TTTCTTGGCA GCATCGCAGT 1900
GCTGAATTTA ATAATATAAT TCCTTCATCA CAAATTACAC AAATACCTTT
AACAAAATCT ACTAATCTTG GCTCTGGAAC TTCTGTCGTT AAAGGACCAG 2000
GATTTACAGG AGGAGATATT CTTCGAAGAA CTTCACCTGG CCAGATTTCA
ACCTTAAGAG TAAATATTAC TGCACCATTA TCACAAAGAT ATCGGGTAAG 2100
AATTCGCTAC GCTTCTACTA CAAATTTACA ATTCCATACA TCAATTGACG
GAAGACCTAT TAATCAGGGT AATTTTTCAG CAACTATGAG TAGTGGGAGT 2200
AATTTACAGT CCGGAAGCTT TAGGACTGTA GGTTTTACTA CTCCGTTTAA
CTTTTCAAAT GGATCAAGTG TATTTACGTT AAGTGCTCAT GTCTTCAATT 2300
CAGGCAATGA AGTTTATATA GATCGAATTG AATTTGTTCC GGCAGAAGTA
ACCTTTGAGG CAGAATATGA TTTAGAAAGA GCACAAAAGG CGGTGAATGA 2400
GCTGTTTACT TCTTCCAATC AAATCGGGTT AAAAACAGAT GTGACGGATT
ATCATATTGA TCAAGTATCC AATTTAGTTG AGTGTTTATC AGATGAATTT 2500
TGTCTGGATG AAAAACAAGA ATTGTCCGAG AAAGTCAAAC ATGCGAAGCG
```

Table 3 (cont.)

```
ACTTAGTGAT GAGCGGAATT TACTTCAAGA TCCAAACTTC AGAGGGATCA 2600
ATAGACAACT AGACCGTGGC TGGAGAGGAA GTACGGATAT TACCATCCAA
GGAGGCGATG ACGTATTCAA AGAGAATTAC GTTACGCTAT TGGGTACCTT 2700
TGATGAGTGC TATCCAACGT ATTTATATCA AAAAATAGAT GAGTCGAAAT
TAAAAGCCTA TACCCGTTAT CAATTAAGAG GGTATATCGA AGATAGTCAA 2800
GACTTAGAAA TCTATTTAAT TCGCTACAAT GCAAAACATG AAACAGTAAA
TGTGCCAGGT ACGGGTTCCT TATGGCCGCT TTCAGCCCAA AGTCCAATCG 2900
GAAAGTGTGG AGAGCCGAAT CGATGCGCGC CACACCTTGA ATGGAATCCT
GACTTAGATT GTTCGTGTAG GGATGGAGAA AAGTGTGCCC ATCATTCGCA 3000
TCATTTCTCC TTAGACATTG ATGTAGGATG TACAGACTTA AATGAGGACC
TAGGTGTATG GGTGATCTTT AAGATTAAGA CGCAAGATGG GCACGCAAGA 3100
CTAGGGAATC TAGAGTTTCT CGAAGAGAAA CCATTAGTAG GAGAAGCGCT
AGCTCGTGTG AAAAGAGCGG AGAAAAAATG GAGAGACAAA CGTGAAAAAT 3200
TGGAATGGGA AACAAATATC GTTTATAAAG AGGCAAAAGA ATCTGTAGAT
GCTTTATTTG TAAACTCTCA ATATGATCAA TTACAAGCGG ATACGAATAT 3300
TGCCATGATT CATGCGGCAG ATAAACGTGT TCATAGCATT CGAGAAGCTT
ATCTGCCTGA GCTGTCTGTG ATTCCGGGTG TCAATGCGGC TATTTTTGAA 3400
GAATTAGAAG GGCGTATTTT CACTGCATTC TCCCTATATG ATGCGAGAAA
TGTCATTAAA AATGGTGATT TTAATAATGG CTTATCCTGC TGGAACGTGA 3500
AAGGGCATGT AGATGTAGAA GAACAAAACA ACCAACGTTC GGTCCTTGTT
CTTCCGGAAT GGGAAGCAGA AGTGTCACAA GAAGTTCGTG TCTGTCCGGG 3600
TCGTGGCTAT ATCCTTCGTG TCACAGCGTA CAAGGAGGGA TATGGAGAAG
GTTGCGTAAC CATTCATGAG ATCGAGAACA ATACAGACGA ACTGAAGTTT 3700
AGCAACTGCG TAGAAGAGGA AATCTATCCA AATAACACGG TAACGTGTAA
TGATTATACT GTAAATCAAG AAGAATACGG AGGTGCGTAC ACTTCTCGTA 3800
ATCGAGGATA TAACGAAGCT CCTTCCGTAC CAGCTGATTA TGCGTCAGTC
TATGAAGAAA AATCGTATAC AGATGGACGA AGAGAGAATC CTTGTGAATT 3900
TAACAGAGGG TATAGGGATT ACACGCCACT ACCAGTTGGT TATGTGACAA
AAGAATTAGA ATACTTCCCA GAAACCGATA AGGTATGGAT TGAGATTGGA 4000
GAAACGGAAG GAACATTTAT CGTGGACAGC GTGGAATTAC TCCTTATGGA
GGAA (end HD-1)
```

Table 3A

Deduced Amino Acid Sequence of Chimeric Toxin

ACB-1

M D N N P N I N E C I P Y N C L S N P E V E V L G G E R I E
T G Y T P I D I S L S L T Q F L L S E F V P G A G F V L G L
V D I I W G I F G P S Q W D A F L V Q I E Q L I N Q R I E E
F A R N Q A I S R L E G L S N L Y Q I Y A E S F R E W E A D
F T N P A L R E E M R I Q F N D M N S A L T T A I P L F A V
Q N Y Q V P L L S V Y V Q A A N L H L S V L R D V S V F G Q
R W G F D A A T I N S R Y N D L T R L I G N Y T D Y A V R W
Y N T G L E R V W G P D S R D W V R Y N Q F R R E L T L T V
L D I V A L F P N Y D S R R Y P I R T V S Q L T R E I Y T N
P V L E N F D G S F R G S A Q G I E R S I R S P H L M D I L
N S I T I Y T D A H R G Y Y Y W S G H Q I M A S P V G F S G
P E F T F P L Y G T M G N A A P Q Q R I V A Q L G Q G V Y R
T L S S T L Y R R P F N I G I N N Q Q L S V L D G T E F A Y
G T S S N L P S A V Y R K S G T V D S L N E I P P Q N N N V
P P R Q E F S H R L S H V S M F R S G F S N S S V S I I R A
P T F S W Q H R S A E F N N I I P S S Q I T Q I P L T K S T
N L G S G T S V V K G P G F T G G D I L R R T S P G Q I S T
L R V N I T A P L S Q R Y R V R I R Y A S T T N L Q F H T S
I D G R P I N Q G N F S A T M S S G S N L Q S G S F R T V G
F T T P F N F S N G S S V F T L S A H V F N S G N E V Y I D
R I E F V P A E V T F E A E Y D L E R A Q K A V N E L F T S
S N Q I G L K T D V T D Y H I D Q V S N L V E C L S D E F C
L D E K Q E L S E K V K H A K R L S D E R N L L Q D P N F R
G I N R Q L D R G W R G S T D I T I Q G G D D V F K E N Y V
T L L G T F D E C Y P T Y L Y Q K I D E S K L K A Y T R Y Q
L R G Y I E D S Q D L E I Y L I R Y N A K H E T V N V P G T
G S L W P L S A Q S P I G K C G E P N R C A P H L E W N P D
L D C S C R D G E K C A H H S H H F S L D I D V G C T D L N
E D L G V W V I F K I K T Q D G H A R L G N L E F L E E K P
L V G E A L A R V K R A E K K W R D K R E K L E W E T N I V
Y K E A K E S V D A L F V N S Q Y D Q L Q A D T N I A M I H
A A D K R V H S I R E A Y L P E L S V I P G V N A A I F E E
L E G R I F T A F S L Y D A R N V I K N G D F N N G L S C W
N V K G H V D V E E Q N N Q R S V L V L P E W E A E V S Q E
V R V C P G R G Y I L R V T A Y K E G Y G E G C V T I H E I
E N N T D E L K F S N C V E E E I Y P N N T V T C N D Y T V
N Q E E Y G G A Y T S R N R G Y N E A P S V P A D Y A S V Y
E E K S Y T D G R R E N P C E F N R G Y R D Y T P L P V G Y
V T K E L E Y F P E T D K V W I E I G E T E G T F I V D S V
E L L L M E E

Table 4

Nucleotide Sequence of Plasmid pSYW1 Encoding Chimeric Toxin SYW1

The nucleotide differences as compared to the sequence shown in Table 1 are underlined at positions 1252, 1319, 1320, 1323, 1324, and 1326; and code for amino acid changes at positions 289, 311, and 313, as shown in Table 4A.

```
                (start HD-73)              ATG GATAACAATC  400
CGAACATCAA TGAATGCATT CCTTATAATT GTTTAAGTAA CCCTGAAGTA
GAAGTATTAG GTGGAGAAAG AATAGAAACT GGTTACACCC CAATCGATAT  500
TTCCTTGTCG CTAACGCAAT TTCTTTTGAG TGAATTTGTT CCCGGTGCTG
GATTTGTGTT AGGACTAGTT GATATAATAT GGGGAATTTT TGGTCCCTCT  600
CAATGGGACG CATTTCTTGT ACAAATTGAA CAGTTAATTA ACCAAAGAAT
AGAAGAATTC GCTAGGAACC AAGCCATTTC TAGATTAGAA GGACTAAGCA  700
ATCTTTATCA AATTTACGCA GAATCTTTTA GAGAGTGGGA AGCAGATCCT
ACTAATCCAG CATTAAGAGA AGAGATGCGT ATTCAATTCA ATGACATGAA  800
CAGTGCCCTT ACAACCGCTA TTCCTCTTTT TGCAGTTCAA AATTATCAAG
TTCCTCTTTT ATCAGTATAT GTTCAAGCTG CAAATTTACA TTTATCAGTT  900
TTGAGAGATG TTTCAGTGTT TGGACAAAGG TGGGGATTTG ATGCCGCGAC
TATCAATAGT CGTTATAATG ATTTAACTAG GCTTATTGGC AACTATACAG 1000
ATTATGCTGT ACGCTGGTAC AATACGGGAT TAGAACGTGT ATGGGGACCG
GATTCTAGAG ATTGGGTAAG GTATAATCAA TTTAGAAGAG AATTAACACT 1100
AACTGTATTA GATATCGTTG CTCTGTTCCC GAATTATGAT AGTAGAAGAT
ATCCAATTCG AACAGTTTCC CAATTAACAA GAGAAATTTA TACAAACCCA 1200
GTATTAGAAA ATTTTGATGG TAGTTTTCGA GGCTCGGCTC AGGGCATAGA
AGGAAGTATT AGGAGTCCAC ATTTGATGGA TATACTTAAC AGTATAACCA 1300
TCTATACGGA TGCTCATAAA GGGGAATATT ATTGGTCAGG GCATCAAATA
ATGGCTTCTC CTGTAGGGTT TTCGGGGCCA GAATTCACTT TTCCGCTATA 1400
TGGAACTATG GGAAATGCAG CTCCACAACA ACGTATTGTT GCTCAACTAG
GTCAGGGCGT GTATAGAACA TTATCGTCCA CTTTATATAG AAGACCTTTT 1500
AATATAGGGA TAAATAATCA ACAACTATCT GTTCTTGACG GGACAGAATT
TGCTTATGGA ACCTCCTCAA ATTTGCCATC CGCTGTATAC AGAAAAAGCG 1600
GAACGGTAGA TTCGCTGGAT GAAATACCGC CACAGAATAA CAACGTGCCA
CCTAGGCAAG GATTTAGTCA TCGATTAAGC CATGTTTCAA TGTTTCGTTC 1700
AGGCTTTAGT AATAGTAGTG TAAGTATAAT AAGAGCT (end hd-73)
    (start HD-1)          CCAACGT TTTCTTGGCA GCATCGCAGT 1900
GCTGAATTTA ATAATATAAT TCCTTCATCA CAAATTACAC AAATACCTTT
AACAAAATCT ACTAATCTTG GCTCTGGAAC TTCTGTCGTT AAAGGACCAG 2000
GATTTACAGG AGGAGATATT CTTCGAAGAA CTTCACCTGG CCAGATTTCA
ACCTTAAGAG TAAATATTAC TGCACCATTA TCACAAAGAT ATCGGGTAAG 2100
AATTCGCTAC GCTTCTACTA CAAATTTACA ATTCCATACA TCAATTGACG
GAAGACCTAT TAATCAGGGT AATTTTTCAG CAACTATGAG TAGTGGGAGT 2200
AATTTACAGT CCGGAAGCTT TAGGACTGTA GGTTTTACTA CTCCGTTTAA
CTTTTCAAAT GGATCAAGTG TATTTACGTT AAGTGCTCAT GTCTTCAATT 2300
CAGGCAATGA AGTTTATATA GATCGAATTG AATTTGTTCC GGCAGAAGTA
ACCTTTGAGG CAGAATATGA TTTAGAAAGA GCACAAAAGG CGGTGAATGA 2400
GCTGTTTACT TCTTCCAATC AAATCGGGTT AAAAACAGAT GTGACGGATT
```

Table 4 (cont.)

```
ATCATATTGA TCAAGTATCC AATTTAGTTG AGTGTTTATC AGATGAATTT 2500
TGTCTGGATG AAAAACAAGA ATTGTCCGAG AAAGTCAAAC ATGCGAAGCG
ACTTAGTGAT GAGCGGAATT TACTTCAAGA TCCAAACTTC AGAGGGATCA 2600
ATAGACAACT AGACCGTGGC TGGAGAGGAA GTACGGATAT TACCATCCAA
GGAGGCGATG ACGTATTCAA AGAGAATTAC GTTACGCTAT TGGGTACCTT 2700
TGATGAGTGC TATCCAACGT ATTTATATCA AAAAATAGAT GAGTCGAAAT
TAAAAGCCTA TACCCGTTAT CAATTAAGAG GGTATATCGA AGATAGTCAA 2800
GACTTAGAAA TCTATTTAAT TCGCTACAAT GCAAAACATG AAACAGTAAA
TGTGCCAGGT ACGGGTTCCT TATGGCCGCT TTCAGCCCAA AGTCCAATCG 2900
GAAAGTGTGG AGAGCCGAAT CGATGCGCGC CACACCTTGA ATGGAATCCT
GACTTAGATT GTTCGTGTAG GGATGGAGAA AAGTGTGCCC ATCATTCGCA 3000
TCATTTCTCC TTAGACATTG ATGTAGGATG TACAGACTTA AATGAGGACC
TAGGTGTATG GGTGATCTTT AAGATTAAGA CGCAAGATGG GCACGCAAGA 3100
CTAGGGAATC TAGAGTTTCT CGAAGAGAAA CCATTAGTAG GAGAAGCGCT
AGCTCGTGTG AAAAGAGCGG AGAAAAAATG GAGAGACAAA CGTGAAAAAT 3200
TGGAATGGGA AACAAATATC GTTTATAAAG AGGCAAAAGA ATCTGTAGAT
GCTTTATTTG TAAACTCTCA ATATGATCAA TTACAAGCGG ATACGAATAT 3300
TGCCATGATT CATGCGGCAG ATAAACGTGT TCATAGCATT CGAGAAGCTT
ATCTGCCTGA GCTGTCTGTG ATTCCGGGTG TCAATGCGGC TATTTTTGAA 3400
GAATTAGAAG GGCGTATTTT CACTGCATTC TCCCTATATG ATGCGAGAAA
TGTCATTAAA AATGGTGATT TTAATAATGG CTTATCCTGC TGGAACGTGA 3500
AAGGGCATGT AGATGTAGAA GAACAAAACA ACCAACGTTC GGTCCTTGTT
CTTCCGGAAT GGGAAGCAGA AGTGTCACAA GAAGTTCGTG TCTGTCCGGG 3600
TCGTGGCTAT ATCCTTCGTG TCACAGCGTA CAAGGAGGGA TATGGAGAAG
GTTGCGTAAC CATTCATGAG ATCGAGAACA ATACAGACGA ACTGAAGTTT 3700
AGCAACTGCG TAGAAGAGGA AATCTATCCA AATAACACGG TAACGTGTAA
TGATTATACT GTAAATCAAG AAGAATACGG AGGTGCGTAC ACTTCTCGTA 3800
ATCGAGGATA TAACGAAGCT CCTTCCGTAC CAGCTGATTA TGCGTCAGTC
TATGAAGAAA AATCGTATAC AGATGGACGA AGAGAGAATC CTTGTGAATT 3900
TAACAGAGGG TATAGGGATT ACACGCCACT ACCAGTTGGT TATGTGACAA
AAGAATTAGA ATACTTCCCA GAAACCGATA AGGTATGGAT TGAGATTGGA 4000
GAAACGGAAG GAACATTTAT CGTGGACAGC GTGGAATTAC TCCTTATGGA
GGAA (end HD-1)
```

Table 4A

Deduced Amino Acid Sequence of Chimeric Toxin SYW1

M D N N P N I N E C I P Y N C L S N P E V E V L G G E R I E
T G Y T P I D I S L S L T Q F L L S E F V P G A G F V L G L
V D I I W G I F G P S Q W D A F L V Q I E Q L I N Q R I E E
F A R N Q A I S R L E G L S N L Y Q I Y A E S F R E W E A D
P T N P A L R E E M R I Q F N D M N S A L T T A I P L F A V
Q N Y Q V P L L S V Y V Q A A N L H L S V L R D V S V F G Q
R W G F D A A T I N S R Y N D L T R L I G N Y T D Y A V R W
Y N T G L E R V W G P D S R D W V R Y N Q F R R E L T L T V
L D I V A L F P N Y D S R R Y P I R T V S Q L T R E I Y T N
P V L E N F D G S F R G S A Q G I E G S I R S P H L M D I L
N S I T I Y T D A H K G E Y Y W S G H Q I M A S P V G F S G
P E F T F P L Y G T M G N A A P Q Q R I V A Q L G Q G V Y R
T L S S T L Y R R P F N I G I N N Q Q L S V L D G T E F A Y
G T S S N L P S A V Y R K S G T V D S L D E I P P Q N N N V
P P R Q G F S H R L S H V S M F R S G F S N S S V S I I R A
P T F S W Q H R S A E F N N I I P S S Q I T Q I P L T K S T
N L G S G T S V V K G P G F T G G D I L R R T S P G Q I S T
L R V N I T A P L S Q R Y R V R I R Y A S T T N L Q F H T S
I D G R P I N Q G N F S A T M S S G S N L Q S G S F R T V G
F T T P F N F S N G S S V F T L S A H V F N S G N E V Y I D
R I E F V P A E V T F E A E Y D L E R A Q K A V N E L F T S
S N Q I G L K T D V T D Y H I D Q V S N L V E C L S D E F C
L D E K Q E L S E K V K H A K R L S D E R N L L Q D P N F R
G I N R Q L D R G W R G S T D I T I Q G G D D V F K E N Y V
T L L G T F D E C Y P T Y L Y Q K I D E S K L K A Y T R Y Q
L R G Y I E D S Q D L E I Y L I R Y N A K H E T V N V P G T
G S L W P L S A Q S P I G K C G E P N R C A P H L E W N P D
L D C S C R D G E K C A H H S H H F S L D I D V G C T D L N
E D L G V W V I F K I K T Q D G H A R L G N L E F L E E K P
L V G E A L A R V K R A E K K W R D K R E K L E W E T N I V
Y K E A K E S V D A L F V N S Q Y D Q L Q A D T N I A M I H
A A D K R V H S I R E A Y L P E L S V I P G V N A A I F E E
L E G R I F T A F S L Y D A R N V I K N G D F N N G L S C W
N V K G H V D V E E Q N N Q R S V L V L P E W E A E V S Q E
V R V C P G R G Y I L R V T A Y K E G Y G E G C V T I H E I
E N N T D E L K F S N C V E E E I Y P N N T V T C N D Y T V
N Q E E Y G G A Y T S R N R G Y N E A P S V P A D Y A S V Y
E E K S Y T D G R R E N P C E F N R G Y R D Y T P L P V G Y
V T K E L E Y F P E T D K V W I E I G E T E G T F I V D S V
E L L L M E E